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Antimicrobial, phytochemical and larvicidal properties of *Jatropha multifida* Linn.

Sillma Rampadarath, Daneshwar Puchooa*, Vijayanti Mala Ranghoo-Sanmukhiya

Department of Agriculture and Food Science, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius

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ABSTRACT

Objective: To evaluate the phytochemical, antimicrobial and insecticidal properties of different extracts of *Jatropha multifida* (*J. multifida*) in Mauritius.
Methods: Qualitative and quantitative methods were used for the determination of the presence of phytochemicals in the crude solvent extracts of *J. multifida*. The antimicrobial sensitivity (disc diffusion method) and antibacterial activity (microdilution method) of 13 microorganisms were reported. The insecticidal properties of the crude solvent extracts were tested against the larvae of two insects, *Bactrocera zonata* and *Bactrocera cucurbitae* (Diptera: Tephritidae), which cause important economic losses to local fruits.
Results: Ethyl acetate was proved to be a good solvent for extraction. *J. multifida* showed very interesting activity against *Bacillus algicola* and *Staphylococcus epidermis*. The plant also showed good larvacidal activity against *Bactrocera zonata*.
Conclusions: This paper reports the dual use of *J. multifida*, hence further studies can be made in term of application.

1. Introduction

The *Jatropha* species are considered as multipurpose, drought resistant, perennial plants belonging to Euphorbiaceae family. They are a tropical plant that can grow in low to high rainfall areas either in the farms as a commercial crop or on the boundaries as a hedge to protect fields from grazing animals and to prevent erosion. They have also lots of importance for the production of biodiesel. However the *Jatropha* species are not limited to only the production of biodiesel. They have multiple industrial applications that can be further exploited. Researchers have been interested in biologically active compounds isolated from plant species against pathogenic microorganisms as an alternative for treating antibiotic-resistant microorganisms[1]. Furthermore, being more health-conscious people are favouring the usage of biopesticide over the traditional pesticides that have proved to have adverse effects on the human health.

Jatropha multifida Linn. (*J. multifida*), commonly called coral bush, is a tree or shrub belonging to the family Euphorbiaceae[2]. *J. multifida* is cultivated in African and Asian countries and the plant has been reported to possess traditional antiseptic properties[3]. *J. multifida* is an uncultivated, non-food wild species which grows around the island of Mauritius as well as in backyards and in the wild. It contains a wide range of phytochemicals, to which its antimicrobial activity is often attributed[4]. This study aims to report the effect of *J. multifida* crude leaves extracts of two different solvents on its ability to hinder growth of microorganisms and its insecticidal properties against *Bactrocera zonata* (*B. zonata*) and *Bactrocera cucurbitae* (*B. cucurbitae*) at larval stage. These two insect larvae cause serious economic losses to the fruit production sector and leguminous plants[5].

*Corresponding author: Daneshwar Puchooa, Department of Agriculture and Food Science Faculty of Agriculture, University of Mauritius, Réduit, Mauritius.
Tel: +2304541041
Fax: +2304655743
E-mail: sudeshp@uom.mu
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2. Materials and methods

2.1. Preparation of plant crude extracts

Three different localities of Mauritius, namely, Curepipe (Central Plateau of Mauritius), Nouvelle France (south of Mauritius) and Grand Baie (north), were chosen for the collection of the mature leaf samples. The plant specimen was sent to the Mauritius Herbarium, Réduit, for identification. The crude extract of the fresh plant materials was prepared by washing under running tap water, air-drying for 3-4 d followed by pounding coarsely. After allowing 20 g of fresh leaves to be macerated for 48 h in 40 mL of two different solvents: methanol and ethyl acetate (AR grade Sigma Chemicals®), the crude extracts were filtered using Whatman filter paper, and pore size was 15-18 μm. Then the extracts were allowed to concentrate in a ventilated fume cupboard at room temperature before storing in the dark bottles at 4 °C for further use.

2.2. Antimicrobial susceptibility tests

A total of 13 microorganisms were tested in this study, including six Gram-positive [*Bacillus algicola* Acc.13/5 (*B. algicola*), *Bacillus cereus* ATCC 11778 (*B. cereus*), *Listeria innocua* ATCC 33090 (*L. innocua*), *Staphylococcus aureus* ATCC 29213 (*S. aureus*), *Staphylococcus epidermis* ATCC 12228 (*S. epidermis*), *Viridibacillus arenosi* strain LMG 22166 (*V. arenosi*)], six Gram-negative [*Escherichia coli* ATCC 25922 (*E. coli*), *Escherichia coli* 0145:H28 Acc. No. CP006027.1, *Klebsiella oxytoca* ATCC 43086 (*K. oxytoca*), *Proteus mirabilis* strain NCTC 11938 (*P. mirabilis*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Salmonella typhimurium* ATCC 14028 (*S. typhimurium*)], and one fungus [*Candida albicans* ATCC 1023 (*C. albicans*)].

Available dehydrated Mueller-Hinton agar (19 g/L, w/v) was prepared for the disc diffusion test. The plates were incubated in an upright position at 37 °C for 24-48 h and zone of inhibition was measured (in mm diameter). One positive control (standard antibiotic tetracycline, 30 μg) and two negative controls (solvents ethyl acetate and methanol) were used.

The serial microdilution method was used to determine the minimum inhibitory concentration (MIC) for antibacterial activity of plant crude extracts. The cultures were standardised by dilution with sterile methemoglobin (0.1 mL inoculum in 9.9 mL methemoglobin) to an absorbance of 0.4-0.6 at 600 nm. About 100 μL of the tested sample for each bacterium was two-fold serially diluted with 100 μL sterile distilled water in a sterile 96-well microplate. A two-fold of tetracycline (30 μg/mL) was also used as positive control. Methanol and ethyl acetate were used as negative control and 100 μL of bacterial suspension was added to each well. The plates were covered and incubated at 37 °C for 24 h, and the lowest concentration of the extract causing complete inhibition of the bacterial growth was noted as MIC. The experiment was performed in triplicates.

2.3. Phytochemical screening

2.3.1. Qualitative and quantitative tests

The tests to detect the presence of flavonoids, alkaloids, saponins, steroids, tannins, coumarins and phenols were carried out. All screenings were based on a series of test tube tests. Phenols and alkaloids were also determined by thin layer chromatography (TLC) (TLC Pre-coated silica gel 60 F_{254} plates Sigma®) and UV techniques[6]. Total flavonoid content (TFC) was determined as quercetin (QE). The total phenolic content (TPC) of plant extract was determined using Folin-Ciocalteu reagent method and was measured as gallic acid (GAE) equivalents (μg/g of fresh weight). The absorbance for both reaction mixes was measured at 510 nm (Jenway spectrophotometer 7305-UV-Visible). A quercetin (0-200 μg/mL) and a gallic acid (0-300 μg/mL) standard curves were plotted for the determination of the flavonoid and phenol content.

2.4. Toxicity assay

B. zonata and *B. cucurbitae* larvae of 3-day-old were collected from the Entomology Division of the Ministry of Agro-Industry and Food Security. The methanolic and the ethyl acetate crude extracts of *J. multifida* were tested for their larvicidal effect on two types of fruit flies larvae at three different doses-0.2, 0.4 and 0.8 mg/L (w/v) extracts. The formulation of natural food diet was sprayed with 10 mL of the different concentration of the plant extracts. The experiment was set in a completely randomised manner with 3 replicates of 10 larvae per treatment (extract) and control (with and without the solvent). The % mortality was recorded at 24, 48 and 72 h.

2.5. Statistical analysis

Mean±SD with One-way ANOVA at 5% was calculated for the phytochemical and antimicrobial assays with least significant difference (LSD) test to compare the differences between the means. The mortality assay data collected were subjected to log (base10) transformation prior to analysis and the lethal doses (LD) were calculated using the probit analysis. All the statistical analyses, and tables and graphs output were done in Minitab® 16.2.4 and Microsoft Excel 2010 software.

3. Results

In ethyl acetate extract of *J. multifida*, alkaloids, phenols, steroids and tannins were present, and coumarins, flavanoids and saponins were absent. While in methanolic extract, alkaloids, flavanoids, phenols and steroids were present, and coumarins, saponins and tannins were absent.

Table 1

MIC of the solvent crude extracts of *J. multifida* against Gram-positive and Gram-negative microorganisms (μg/mL).

Solvents	Gram-positive						Gram-negative						
	1	2	3	4	5	6	7	8	9	10	11	12	13
Methanol	100.00	100.00	10.88	100.00	14.50	50.00	2.70	3.60	100.00	37.50	100.00	100.00	25.00
Ethyl acetate	25.00	50.00	7.25	100.00	25.00	75.00	7.25	37.50	37.50	75.00	19.75	14.50	14.50
Tetracycline (30 μg/mL)	0.90	3.60	30.00	11.25	22.50	15.00	1.80	1.80	30.00	3.70	1.80	11.13	100.00

1: *B. algicola* Acc. No. 13/5; 2: *B. cereus* ATCC 11778; 3: *L. innocua* ATCC 33090; 4: *S. aureus* ATCC 29213; 5: *S. epidermis* ATCC 12228; 6: *V. arenosi* strain LMG 22166; 7: *E. coli* ATCC 25922; 8: *E. coli* 0145:H28 Acc. No.CP006027.1; 9: *K. oxytoca* ATCC 43086; 10: *P. mirabilis* strain NCTC 11938; 11: *P. aeruginosa* ATCC 27853; 12: *S. typhimurium* ATCC 14028; 13: *C. albicans* ATCC 1023.

3.1. Quantification of TPC and TFC

The content of phenol in the crude extract was about 30 fold higher to the content of the flavonoid (Figure 1).

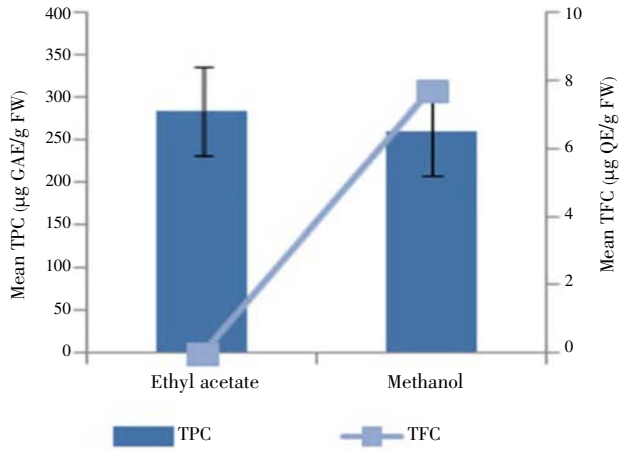

Figure 1. Quantification of the TFC and TPC in the crude plant solvent extracts. One-way ANOVA with LSD at 5%, n=5.

3.2. Antimicrobial assay

3.2.1. Antibacterial and antifungal susceptibility test

The mean zone of inhibition of the crude extract for the tested microorganisms is depicted in Figure 2.

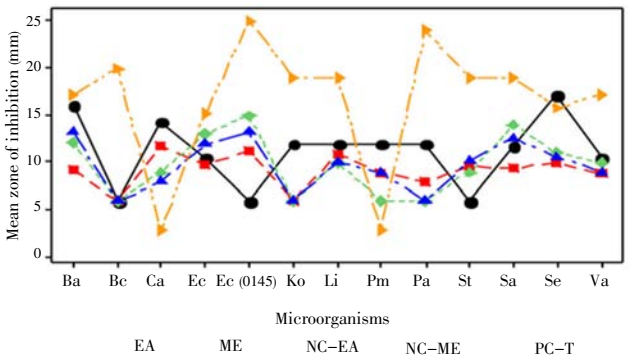

Figure 2. Mean zone of inhibition of solvent crude extracts of *J. multifida* against different microorganisms.
EA: Ethyl acetate; ME: Methanol; PC-T: Positive control (tetracycline); NC: Negative control. Ba: *B. algicola*; Bc: *B. cereus*; Ca: *C. albicans*; Ec: *E. coli*; Ec (0145): *E. coli* (0145); Ko: *K. oxytoca*; Li: *L. innocua*; Pm: *P. mirabilis*; Pa: *P. aeruginosa*; St: *S. typhimurium*; Sa: *S. aureus*; Se: *S. epidermis*; Va: *V. arenosi*. One-way ANOVA with LSD at 5%, n=5.

3.2.2. Antimicrobial activity (MIC)

The MIC of the crude extract is tabulated in Table 1.

3.3. Crude solvent extracts: toxicity bioassay against Bactrocera sp.

The lethal doses at 50% and 90% of the leaf extracts against the diptera larval stage are shown in Table 2. The mean % mortality of both *B. cucurbitae* and *B. zonata* at larval stage for the different of concentration of crude solvent extracts with time is shown in Figure 3.

Table 2

Lethal dose (LD) of the two crude solvent extracts against diptera species.

Diptera	Extracts	Lethal dose (g/L)	
		LD_{50}	LD_{90}
B. cucurbitae	ME	0.36	1.86
	EA	0.35	1.00
B. zonata	ME	0.17	0.66
	EA	<0.01	<0.01

No. of larvae/treatment (n=10) are in triplicates. EA: Ethyl acetate; ME: Methanol. LD_{50}/LD_{90}: Dose level at which 50% and 90% larvae death (probit analysis).

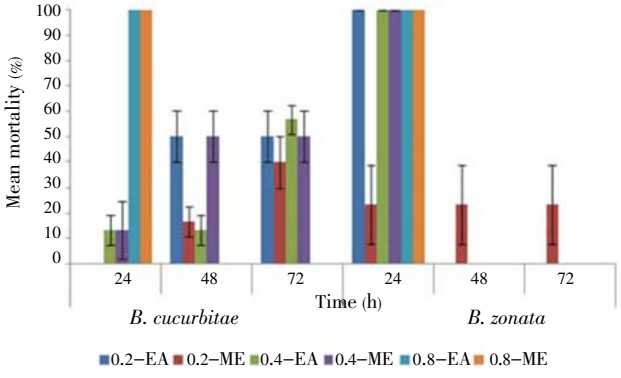

Figure 3. Mean mortality (%) of both *B. cucurbitae* and *B. zonata* at larval stage for the different of concentration of crude solvent extracts with time. Data are expressed as mean±SD with LSD at 5%, n=10. Controls: For solvents with or without, no death of the larvae were observed.

4. Discussion

Phytochemical and fractions (TLC) screening initially revealed the presence of alkaloids, flavonoids, steroids

tannins, and phenols in the two crude solvent extracts. The retention factor (R_f) value of 0.42 for the methanolic extract of *J. multifida* showed the presence of flavonoid-glycoside compound. Both the methanolic and ethyl acetate extracts gave R_f values of 0.72 and 0.74 respectively for phenols screening.

The two solvents used did have an effect on the result obtained for the phytochemical screenings. This is in line with some findings that had been reported[7] about different solvents having different spectrum of solubility for the phytoconstituents. Flavanoids presence was obtained only for methanolic crude extract of *J. multifida*. The presence of alkaloids, steroids and phenols were not dependent on the two solvents used as they were present in both extracts. Spots at different distance on the TLC plates and the computed R_f values indicated the presence of different flavonoids in the methanolic extract and phenolic compounds in the leaves crude extracts. *J. multifida* leaves have been found to contain saponin in its aerial part, however in this study saponin was not detected.

The TPC and TFC of the two solvent crude extracts showed significantly differences ($P<0.05$) for the different medicinal plants. The quantitative analysis of the phytochemicals revealed that the total phenol content was higher compared to the flavonoid content and that the methanolic crudes extracts of mature leaves yielded both phenol (260.67±51.94 μg GAE/g) and flavonoid (7.70±0.19 μg QE/g).

Significant varying degrees of antibacterial and antifungal potential ($P<0.05$) were obtained for the two crude extracts. The antibacterial activity of all extracts depends largely upon the type of solvent used for extraction and the bacterial strains tested in the susceptibility assay. The crude extract from leaves of *J. multifida* showed the highest antibacterial activity against Gram-positive strains with a mean zone of inhibition of (17.20±1.79) mm diameter against *S. epidermis* (ATCC 12228) and (16.00±1.00) mm diameter for *B. algicola* Acc. 13/5. An observation is worth reporting as it has been previously highlighted that *J. multifida* does not have any antimicrobial activity against Gram-positive bacteria[4,7].

The crude ethyl acetate and the methanolic extracts also showed antifungal properties against *C. albicans* (ATCC 1023). Moreover, the antimicrobial activity of the ethyl acetate crude extracts seemed to be more effective than the methanolic crude extracts for inhibiting microorganism activity for both Gram-positive and Gram-negative strains[1,3].

The effect of the different concentrations (LD) of the crude extracts varied significantly on the two diptera larvae. The crude ethyl acetate extract of *J. multifida* demonstrated greater efficiency for larval control of *B. zonata* with LD_{90} of less than 0.01 g/L. The 100% mortality was also noted for *B. cucurbitae* for crude methanolic extract of *J. multifida* at a dose of 0.8 g/L.

The present study indicates that *J. multifida* has both antimicrobial as well as larvicidal properties. Further research should be aimed at identification of bioactive compounds that can be used as antibiotics and/or insecticides.

Conflict of interest statement

We declare that we have no conflict of interest.

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